

The University of Chicago

AN ANALYSIS OF THE PROCESS OF RE-
GENERATION IN CERTAIN MICRO-
DRILOUS OLIGOCHAETES

A DISSERTATION

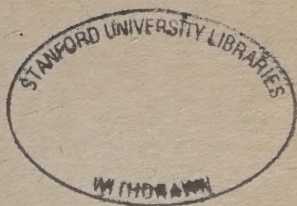
SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL
OF SCIENCE IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF ZOÖLOGY)

BY

LIBBIE H. HYMAN

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AN ANALYSIS OF THE PROCESS OF REGENERATION IN CERTAIN MICRODRILOUS OLIGOCHAETES

LIBBIE H. HYMAN
Hull Zoölogical Laboratory

TWENTY-FOUR FIGURES

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The importance of regeneration as an experimental method has not been sufficiently recognized. The numerous investigations which have been carried on in this field have given us a vast amount of data regarding the capacity for regeneration of all groups of animals, the axial differences in rate and amount of regeneration, the histology of the process, and so on, but little progress has been made in the interpretation and analysis of the facts observed. Child ('11 d) has pointed out the fundamental biological significance of regulatory phenomena, and has shown

that important problems may be attacked by means of this relatively simple experimental method.

Under Professor Child's direction, I have been carrying out experiments along similar lines on several species of microdrilous oligochaetes; this paper is a partial report of the results of these investigations.

I. MATERIAL

The following species have been used in these experiments:

Aeolosomatidae

Aeolosoma hemprichii Ehrenberg.

Naididae

Dero limosa Leidy.

Dero furcata Oken.

Stylaria lacustris Linnaeus.

Chaetogaster diaphanus Gruithuisen.

Nais elinguis Müller.

Lumbriculidae

Lumbriculus inconstans Smith.

Tubificidae

Tubifex tubifex Lamarck.

Limnodrilus claparedianus Ratzel.

Aeolosoma was obtained from ordinary Protozoan cultures such as are made up for class use. The naids were collected from old ponds and streams, especially Wolf Lake, Indiana, and the Des Plaines River near Lyons, Illinois; quantities of mud and vegetation were brought in from such places, and put into large crystallizing dishes. I have not found it practicable to keep any naids but *Dero* for any length of time in the laboratory, but this form is readily cultivated, and thrives and reproduces rapidly if a small amount of fermenting grain is added to the culture. The tubificids were collected in the same places and in the same way as the naids; they can be kept for months under laboratory conditions, and are also benefited by the addition of grain to the culture. *Lumbriculus inconstans*, unlike the other fresh-water oligochaetes, is very restricted as to habitat, occurring, in the Chicago vicinity at least, only in temporary

forest pools of a characteristic ecological type (see Shelford, '14, pp. 179, 185). This species is active, therefore, only during the spring months when the pools contain water, since it passes into an encysted condition when the water evaporates with the approach of summer.¹ Mrazek ('13) states that such pools form also the typical habitat of *Lumbriculus variegatus*, and his further remarks on the ecology of this species apply equally well to *Lumbriculus inconstans*.

II. THE AXIAL GRADIENT

1. *The cyanide method*

The most important general fact which has come out of all the work on regeneration is this;—that the result differs according to the level along the antero-posterior axis at which section is made. This difference may be one of rate or of amount of new tissue formed, or, in the most interesting cases, the structure which appears at the cut surface varies according to the level of section. Such an axial difference is obviously the expression of a preexisting internal gradient of some sort,—a protoplasmic gradient independent of the more obvious morphological features of the organism. This gradient must undoubtedly have both structural and functional components, for structure and function always interact; in the living organism they can have no separate existence. But it is the functional component alone with which I propose to deal in this discussion of the axial gradient.

What means have we for demonstrating a functional, dynamic gradient—or, to put it more simply, a gradient in metabolic processes—along the axis of the living organism? Child ('13 a) has devised and extensively employed a simple method depending on the use of lethal concentrations of anaesthetics and cyanides. In the presence of such substances—and of many other poisons also—metabolism cannot continue, and if there exists a metabolic gradient in the organism, its parts will show a different

¹ A complete account of the life cycle of *Lumbriculus inconstans* in relation to its peculiar habitat will be published elsewhere.

degree of susceptibility, or, conversely, a different degree of resistance to the action of these substances. This will be especially true if the metabolic difference is one of rate, for those parts in which metabolism is going on most rapidly must be most susceptible to substances which stop metabolism; moreover, we have no reason to assume that the fundamental metabolic processes are different in kind along the axis of a simple organism. The method is, therefore, within certain limits, specifically a method for demonstrating differences in metabolic rate between the parts of an organism, or between comparable organisms.

Regarding the exact way in which substances containing the cyanogen radical and anaesthetics act on protoplasm, there is at present much difference of opinion. It would be fruitless to review here the numerous theories which have been advanced with regard to the action of anaesthetics (Gwathmey, '14, Chap. II) among them probably the Verworn conception of anaesthesia as an asphyxiation has been received with the most favor. The most recent evidence does not, however, support this view. Thus Loeb and Wasteneys ('13 a, '13 b) and Winterstein ('14) have measured the oxygen consumption under anaesthesia, and have found that narcosis may occur without any decrease in oxygen consumption, or usually only a slight one, or even a slight increase. Winterstein ('13, '14) also calls attention to many other data leading to the conclusion that the depression of oxidation is not the primary factor in the production of anaesthesia, as Verworn maintains. On the other hand, Tashiro and Adams ('14) have reported a decided decrease in CO_2 production in the anaesthetized nerve of the spider crab. Further experimental work is much needed before any conclusion regarding the nature of anaesthesia can be drawn.

More agreement exists as to the effect of the cyanides on living matter. Since the work of Geppert, it has been generally accepted that the cyanides act by diminishing or inhibiting the oxidation processes. Geppert ('89) showed that an animal poisoned by hydrocyanic acid was consuming less than the usual amount of oxygen, even though such an animal goes into violent convulsions; and further, that the oxygen content of the venous

blood in cyanide poisoning is abnormally high. Geppert therefore concluded that cyanogen reduces the capacity of the cells to use oxygen. This conclusion is supported by the experiments of Warburg ('10), of Loeb ('06 a, '06 b, '10 a, '10 b), and of Loeb and Wasteneys ('10, '11, '13 a, '13 b), who found that the oxygen consumption of eggs and other cells is decreased in the presence of cyanides; of Schroeder ('07) who obtained the same result with *Aspergillus*; and of Vernon ('06) who showed that the oxygen consumption of a perfused kidney is diminished when cyanides are present in the perfused fluid; and I myself, working with sponges, have recently demonstrated to my entire satisfaction that the oxygen consumption of these animals is invariably lowered when potassium cyanide is present in the sea water, even in concentrations as low as $\frac{N}{25000}$.² The cyanides also have a general depressing effect on enzymatic processes, and on many chemical actions, oxidative and otherwise. On the other hand, there have not been wanting objections to the idea that the cyanides inhibit oxidations; principal among these is the statement that the cyanides are equally poisonous to tissues and organisms which are not affected by the absence of atmospheric oxygen, as the nerve cord of the *Limulus* heart (Carlson, '07), and anaerobic bacteria. In reply to this, it may be suggested that the series of chemical processes which we call oxidation is probably much the same up to a certain point in both aerobic and anaerobic organisms, and it is these initial reactions which the cyanide affects (Matthews, '05).

Direct evidence that the susceptibility to cyanide runs parallel with the rate of metabolism has been furnished by Child ('13 a), who has experimentally determined the following facts:

1. Animals stimulated to motor activity are more susceptible than quiescent ones.
2. The susceptibility increases with rising temperature; and the temperature coefficient of susceptibility is the same as the usual temperature coefficient for chemical reactions in general.
3. Other forms of stimulation (as injury, cutting the animal into pieces) increase the susceptibility to cyanide.

² These results will be published shortly.

4. Young animals are invariably more susceptible to cyanide than old ones.

5. The CO_2 production of pieces or animals runs parallel with their susceptibility to cyanide, *i.e.*, those more susceptible to cyanide show also a more rapid CO_2 production. The CO_2 production was determined by Dr. Tashiro with his very ingenious apparatus for measuring minute amounts of carbon dioxide (Tashiro, '13).

According, then, to the facts here presented, the time of death in cyanide bears a direct relation to the previous rate of metabolism. Individuals or parts with the highest rate of metabolism die first, those with the lowest rate last, and the others at intermediate times; the cause of this, as already stated, is to be sought in the asphyxiating action of the cyanides, as a result of which the time of death of each part is proportional to its rate of oxygen consumption. It is only necessary that the death point should be clearly indicated to the observer; this is brought about in the lower invertebrates through the disintegration which promptly follows death. As a check on one's observations, one may remove the animal or piece at any stage of the disintegration to water, whereupon recovery of the intact parts takes place. In this way, I have satisfied myself that disintegration follows death almost instantly. The cyanide method is, of course, not applicable to forms in which, owing to resistant outer structures, disintegration cannot occur; but even in such forms, the death of the animal as a whole may usually be determined by employing some other criterion of the death point.

The technique of the cyanide method is simple. A concentration of potassium cyanide sufficient to kill the animals within one to three hours is used. This concentration must be determined for each species by preliminary experiments. For the oligochaetes it varies from $\frac{1}{50}$ to $\frac{1}{1000}$ normal. The cyanide solution is made up fresh by weight for each experiment. Animals or pieces which are to be compared as to susceptibility must have been kept under the same conditions of food, temperature, etc., previous to the experiment, and must be of approximately the same size, unless size differences are the object of the experiment.

Wherever susceptibilities are compared, I have always done so at the same time, and with the same cyanide solution, thus avoiding sources of error arising from differences in the solution, external conditions, etc. I have found it most convenient to carry out the experiments in watch glasses. The animals are placed in these, freed as much as possible from water, and a cover put on in such a way as to exclude all air bubbles. In such covered watch glasses evaporation of the cyanide is reduced to a minimum, and the whole can be placed under the low power of the compound microscope, and the progress of the disintegration followed very exactly.

The following changes take place in cyanide. The worms at first move about vigorously but eventually pass into a state of anaesthesia. The peristalsis of the intestine and especially of the dorsal blood vessel keeps up as a rule until the time of death. Frequently there is a swelling of the animal, due to the intake of fluid into the coelomic spaces, so that the body wall is distended, except at the septa, and the animal then resembles a string of beads. This condition appears to be a regular ante-mortem change since it also occurs in pieces dying in water. The death point is characterized by an abrupt change from the normal yellowish-red color of the oligochaetes to an opaque white; this change of color is quite apparent to the naked eye, and under the microscope can be followed from segment to segment. Simultaneously with or immediately following this alteration of color, the body wall breaks (it may previously have shown blister-like elevations), and all the structures disintegrate into a shapeless mass of granules. This disintegration, as already indicated, does not occur simultaneously throughout the animal, but proceeds in a perfectly definite manner along the antero-posterior axis. This disintegration gradient of the various oligochaetes will now be described in detail.

2. The primary gradient

The kind of gradient which Child has described for Protozoa, Coelenterates and flatworms (Child, '13 c, '14 a, '14 b) constitutes what I call here the primary gradient. In these forms, disinte-

gration begins at the anterior end, and proceeds backwards along the axis to the posterior end. This is interpreted to mean that the head, or what represents the head, of the organism is carrying on metabolic processes at the highest rate, and that the rate of metabolism decreases along the antero-posterior axis. This kind of gradient exists in the egg, and Child ('13 c) has suggested that it is the physiological basis for the law of antero-posterior development. Child further suggests that this gradient, existing in the protoplasm of the egg is carried over to the nervous system when the latter develops. The cephalic end of the nervous system thus exercises from the very first physiological dominance over the rest of the organism, and retains this dominance by virtue of its functional relations to other parts, even though its actual metabolic rate may, and does, fall throughout ontogeny. The gradient of the adult animal may therefore vary markedly from the original gradient; various parts may attain a higher rate of metabolism than the head itself; but the nervous system, owing to long-established antero-posterior conduction paths, can maintain control, for some time at least, over regions of higher metabolic activity than its own.

Is the gradient of the adult oligochaete of the primary type? I have found it so in but one species examined, namely, *Aeolosoma hemprichii*, a member of the most primitive family of oligochaetes. This worm is very small, only 1-2 mm. in length, has a rounded prostomium, a ciliated funnel-shaped pharynx leading into the intestine, and numerous red oil globules in the body wall. Each animal nearly always consists of two or more zooids which arise in connection with fission planes in the typical annelid manner; before they appear morphologically, they are present physiologically, as is readily demonstrated by the cyanide method.

For the disintegration experiments, a concentration of $\frac{N}{100}$ KCN is used, and the animals are placed in covered watch glasses as already described. In an individual without zooids, the disintegration begins at the tip of the prostomium and proceeds at first slowly, then more rapidly along the axis. The integumental oil globules remain intact longer than the parts in which they were imbedded, but eventually they vanish by sudden extrusion

of their colored contents. The gradient of such an individual is graphically depicted in text figure 2. In these graphs the abscissae represent the number of segments and the ordinates, the time of death in minutes. The dots along the curve are the points actually determined experimentally. In text figure 2, the flatness of the curve between the third and the fourth segments indicates that the head of a zooid is forming there; this graph should therefore be compared with figure 1, which is the disintegration gradient of a fully developed posterior zooid of *Aeolosoma*, and which illustrates a typical primary gradient—

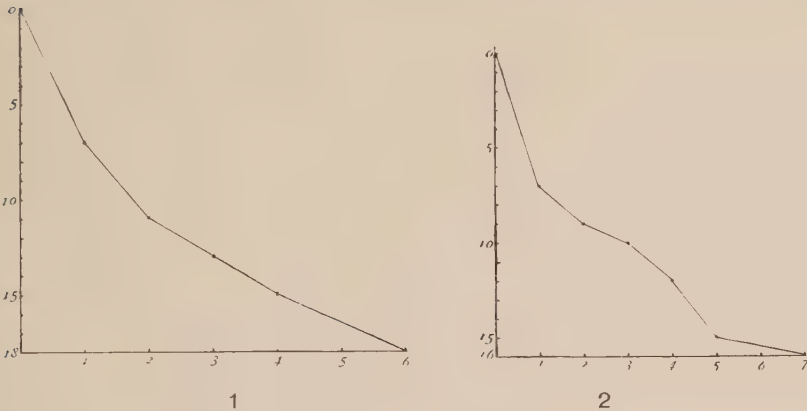


Fig. 1 The axial gradient of a mature zooid of *Aeolosoma hemprichii*, illustrating an ideal primary gradient.

Fig. 2 The axial gradient of an individual *Aeolosoma* in which physiological isolation of a zooid is beginning.

i.e., one that is steepest at the anterior end, and gradually falls off posteriorly.

As the individual *Aeolosoma* grows, the posterior zooid continues to differentiate physiologically. When such an individual is allowed to disintegrate in cyanide, the first change that occurs is the appearance of a constriction near the posterior end. This constriction marks the position of the head of the posterior zooid. Disintegration then proceeds independently in both zooids, from the anterior to the posterior end of each. Four stages in the disintegration of such an individual are illustrated in figure 3,

and a graph of the same in figure 4. The relative time of disintegration of the second zooid as compared with the first depends



Fig. 3 Three stages in the disintegration of an *Aeolosoma* in which physiological isolation of a zooid is well advanced.

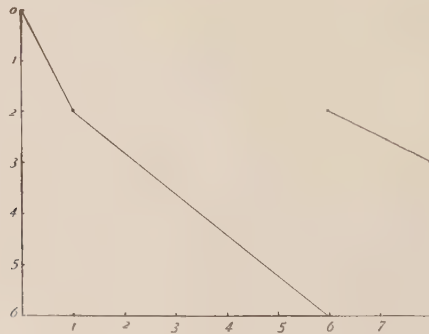


Fig. 4 Graph of the axial gradient of an individual similar to figure 3.

on the degree of development of the former; at the beginning of its existence, when it is present physiologically only, its time

of death about coincides with that of the principal zooid; but, owing to the processes of dedifferentiation and growth involved in its formation, its susceptibility to cyanide continually increases so that its death comes to preceded by a considerable time interval that of the principal zooid. The rate of metabolism of the zooid thus continuously rises during its development.

Eventually the presence of the posterior zooid is made known through the appearance of a fission plane, and its structural

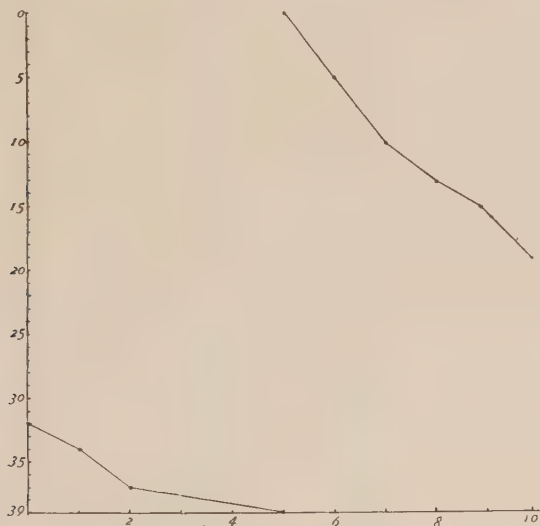


Fig. 5 Graph of an *Aeolosoma* with a zooid well differentiated morphologically, showing the two independent gradients, that of the zooid being at a higher level.

differentiation proceeds. If examined in cyanide at this time, the two independent gradients show very clearly, that of the posterior zooid being at a higher level (fig. 5). The axial gradient of the posterior zooid is of the primary type, and remains so, indeed, until it has separated from the parent animal and begun to produce zooids of its own. The gradient of the anterior zooid is at the beginning of the development of the posterior zooid also of the primary kind but later becomes altered. Disintegration starts at its head and proceeds posteriorly, but soon the

posterior end begins to disintegrate, and this disintegration proceeds anteriorly. The two waves of disintegration meet somewhere about the middle of the zooid. The interpretation of this sort of gradient is simple. Annelids grow characteristically by the formation of new segments in front of the anal segment;

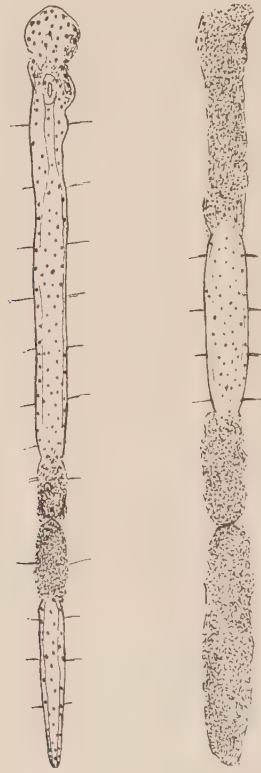


Fig. 6 Two stages in the disintegration of an Aeolosoma with a zooid, showing the secondary posterior rise in the principal zooid.

the new segments thus formed would be expected to have a high rate of metabolism, and therefore an increased susceptibility to cyanide. As soon as the Aeolosoma individual has formed a posterior zooid, it begins to grow in this fashion at its new posterior end, and this growing region shows increased susceptibility to cyanide (fig. 6). In fact, this process may go to such an

extent that the axial gradient of the anterior zooid is reversed (fig. 7); here disintegration begins at the posterior end and proceeds to the anterior end. This condition is found in individuals in which the posterior end has grown considerably without becoming physiologically isolated as a zooid; in such cases, the increasing youth of the segments posteriorly, as well as the fall in rate of metabolism of the anterior end through senescence contribute to cause the reversal of the gradient. As soon as the posterior end of such an individual becomes isolated as a zooid, the primary gradient reappears in it.

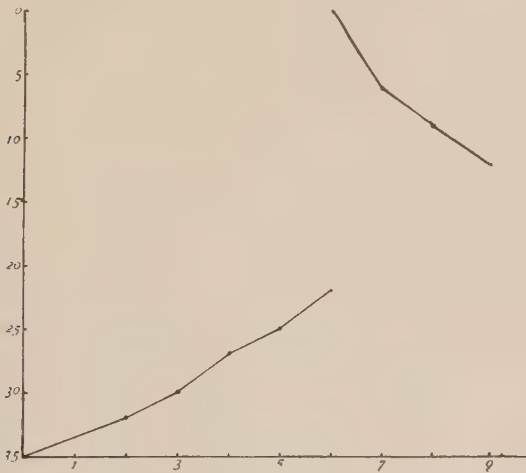


Fig. 7 Graph of an *Aeolosoma* with a zooid, showing reversal of the gradient in the principal zooid.

As a further interesting detail, I may add that although the disintegration of the head usually begins at the tip of the prostomium, yet sometimes, and especially in old individuals, the first region of the head to disintegrate is the ciliated pharynx. Such a specialized organ by virtue of its sensory functions, ciliary activity, etc, must possess a relatively high rate of metabolism which it retains when the rate of surrounding parts has diminished through senescence. Such specialized parts with high susceptibility to cyanide are frequently met with in disintegration

experiments; as examples may be mentioned the sensory auricles of *Planaria*, and the lower lip of the oligochaetes.

Summarizing these results, it is shown that in *Aeolosoma* the primary gradient is present, i.e., the rate of metabolism—as measured by degree of susceptibility to cyanide—is highest at the head, and decreases along the antero-posterior axis. This gradient is present in the zooid from the very beginning of its existence, and continues during its differentiation, and after it has separated. Its rate of metabolism continually increases during this period, but after it has separated and begun to form zooids of its own, the rate falls, and may eventually be exceeded by that of its growing posterior end.

3. *The gradient of the naids*

The primary gradient which exists in *Aeolosoma* is not retained in any other oligochaetes which I have examined except in the zooid stages. I have already spoken in the case of *Aeolosoma* of the rise in susceptibility at the posterior end owing to the formation of new segments there. *Aeolosoma*, however, gives rise to zooids so rapidly that this growing posterior region does not attain to any considerable size because it is always being cut off by zooid formation. But in other Oligochaetes, especially those which do not reproduce asexually at all, this posterior growth is extensive, and has a marked effect on the axial gradient.

Among the naids, I have worked for the most part with *Dero limosa*. A concentration of KCN of $\frac{N}{400}$ or $\frac{N}{500}$ was used. In individuals without fission planes the process of disintegration is as follows. Disintegration begins at the anterior end, involving the tip of the prostomium and the sensory region about the mouth first, and passes posteriorly along the axis; after it has progressed some distance, which varies with individuals, disintegration begins at the posterior end and proceeds forwards; the two waves of disintegration meet about the middle or behind the middle of the worm, the exact point also varying with individuals.

This is the typical annelid gradient,—i.e., one in which the rate of metabolism decreases from the head backwards and rises again at the posterior end.³ This gradient, as already stated, is due to the characteristic method of growth of annelids by formation of new segments posteriorly. If the posterior end has been growing very rapidly, as it does when there is abundant food supply, its rate of metabolism may be higher than that of the head, and it may, therefore, disintegrate first, but the disintegration of the head always follows shortly after.

Three stages in the disintegration of *Dero limosa* are illustrated in figure 8, and a graph of the same in figure 9. In this graph, and in the succeeding ones, I have attempted to compensate for the continual decrease in size of the posterior segments by gradually increasing the number of segments per unit of the cross-section paper. In this way, a truer picture of the gradient is obtained.

The posterior disintegration may begin with the anal segment, or in the region just anterior to this where the youngest segments are forming. In the latter case, the anal segment, which in the

³ As a matter of fact, Morgan ('04), if he had only known it, long ago demonstrated the typical annelid gradient in the earthworm by means of a galvanometer. He found that in the earthworm the anterior and posterior ends are electronegative to the middle part. It is a familiar physiological fact that stimulated regions (i.e., regions of increased metabolism) are electronegative to non-stimulated ones (current of injury, negative variation, electrical variation during the heart beat, etc). The fact that cutting is a stimulation also accounts for the general result of Morgan that cut surfaces of the earth worm are electronegative to intact ones. The results of Morgan also indicate that the clitellum and the fifteenth segment are local regions of high metabolic activity, probably owing to their secretory nature. The data which seemed inexplicable to Morgan therefore find easy explanation when the nature of the annelid gradient and the stimulating effect of cutting are known. There can be no possible doubt that the difference of potential between the intact and the cut surfaces has a different value when the cut surface is to form a head than when it is to form a tail, but there is no reason whatever for assuming that a reversal of potential should occur in the tail forming region of the earthworm. In experiments of this kind there are many important factors to be considered, such as the length of time after cutting, length of the piece, part of the animal from which the pieces are cut, part of the intact surface to which the other electrode is applied, etc. The observations of Czwiklitzer (Arch. f. Entw'mech, Bd. 19) on the disintegration and death of the polychaete *Ophyotrocha* also constitute a demonstration of the annelid gradient.

genus *Dero* is enlarged to form a gill pavilion, remains alive longer than any other part of the body. The anal segment is, of course, one of the oldest parts of the body in Annelids, but in forms which divide by fission, each anterior zooid has to form a new anal segment, while the posterior zooid retains the old anal

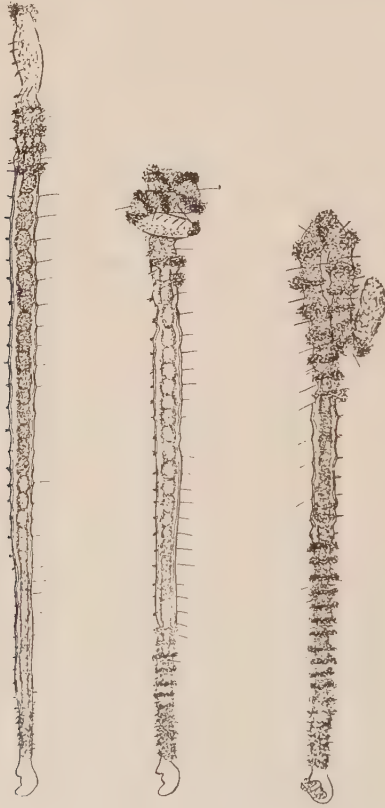


Fig. 8 Three stages in the disintegration of *Dero limosa*.

segment. It thus happens that in any individual *Dero*, the anal segment may be very old, or it may be relatively young, and this accounts for differences in the time of disintegration of this part as compared with the rest of the body.

The axial gradient of *Dero* is modified by the presence of zooids. When the worms have reached a length of 10–15 mm. with 50–80

segments, a fission plane in the form of a constriction appears posterior to the middle of the body. This constriction is coincident with a septum; the anterior end of the new zooid arises between this septum and the succeeding bundles of setae, and the region between the septum and the preceding bundles of setae develops a new posterior end for the old animal. All structures develop completely before separation of the zooids takes place. Usually but two zooids are present. In *Dero*, the region where the fission plane is to appear is not detectable by an increased

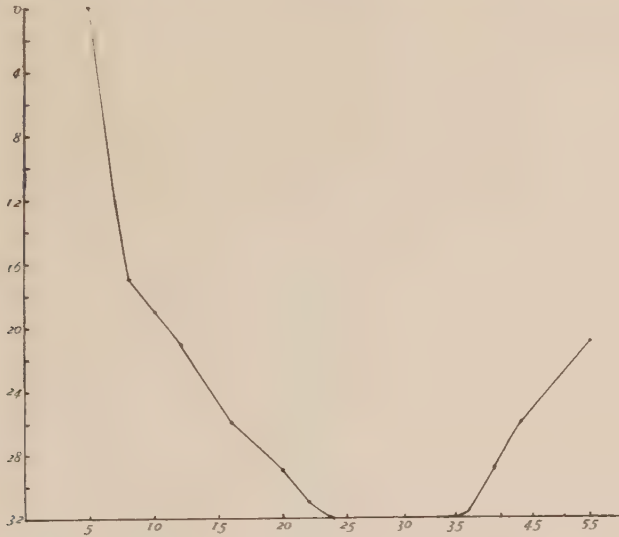


Fig. 9 Graph of the axial gradient of *Dero limosa*, showing the secondary posterior rise typical of oligochaetes.

susceptibility to cyanide, as is the case in *Aeolosoma*. In fact, even after the fission plane is visible, there is no increased susceptibility at that point. It is only after differentiation has begun on the two sides of the fission plane that any alteration of susceptibility is noticeable. At first this consists in a slight increase in susceptibility at the anterior end of the second zooid—that is, disintegration begins at the anterior end of the first zooid, proceeds back some distance, then attacks the anterior end of the second zooid, then the posterior end, and finally the remaining

parts of both. In terms of metabolism, the metabolic rate decreases from the head of the anterior zooid backwards, rises at the head of the second zooid, falls again, and finally rises steadily to the posterior end. Figure 10 illustrates a stage in the disinte-

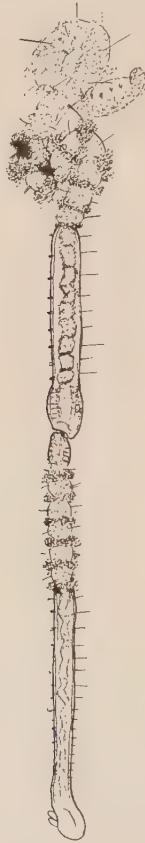


Fig. 10 A stage in the disintegration of a *Dero* with a well developed posterior zooid.

gration of the two zooids, and figure 11 is the corresponding graph. As the development of the head of the posterior zooid proceeds, its susceptibility to cyanide continually increases, approaches that of the head of the first zooid, and eventually exceeds it. Meantime, processes of reorganization have been going on

in the posterior zooid, so that a new gradient is established; this gradient is of the primary type. If, therefore, a worm with a well-developed posterior zooid is allowed to disintegrate in cyanide, it is found to consist of two independent gradients; disintegration begins at the anterior end of each zooid, and proceeds to the posterior end of each (fig. 12). There may be a slight increased susceptibility at the posterior end of the first zooid. After separation of the zooids, posterior growth sets in, producing

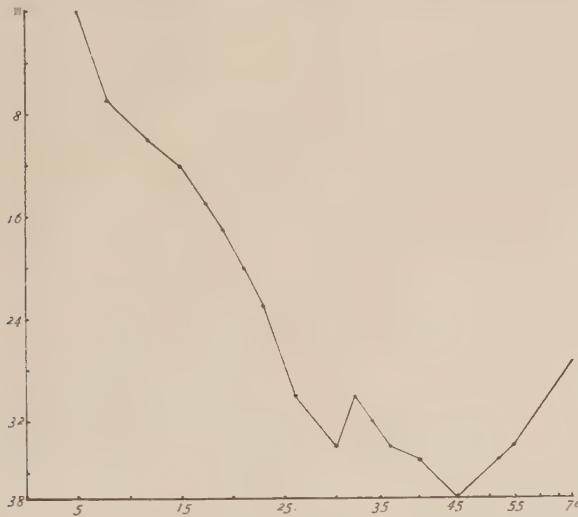


Fig 11 Graph of the axial gradient of *Dero limosa* with a young zooid.

again the rise in rate at the posterior end, which is characteristic of Annelids.

It is to be noted that it is the new anterior end and not the new posterior end in which the marked rise in the rate of metabolism occurs. This change in rate is connected, first, with the formation of a new head, and, secondly, with the reorganization of the parts behind this head to form a new individual. The visible changes in this latter process consist in the working over of the anterior part of the intestine to form an oesophagus, and of the circulatory system to form the 'hearts.' As a result of these changes, the primary gradient is restored.

The axial gradient of other naids is similar in all respects to that of *Dero limosa*, and the changes connected with zooid formation are also the same. I have examined *Stylaria lacustris* in some detail with regard to the gradient, and *Dero furcata*, and *Nais elinguis* less thoroughly, and these forms agree completely with *Dero limosa*. I have also performed some disintegration experiments with the marine syllid, *Autolytus cornutus*, which also reproduces asexually, and whose gradient is quite like that

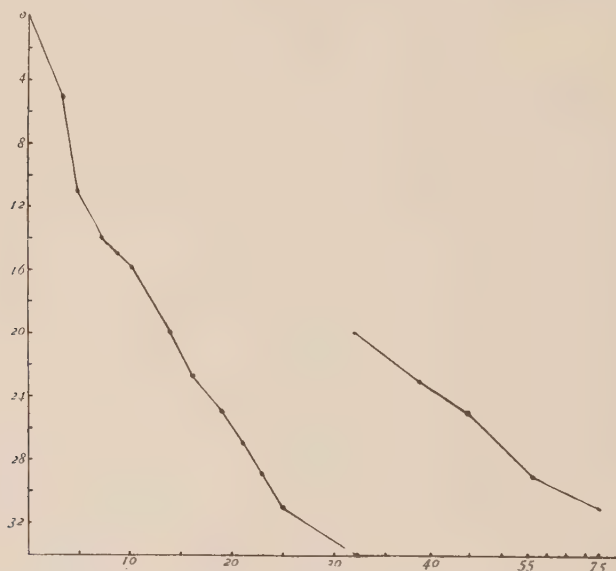


Fig. 12 Graph of the axial gradient of *Dero limosa* with a well-developed zooid, showing the two independent gradients.

of the naids. The polychaetes, are however, unfavorable for this kind of experiment because they do not disintegrate readily. The gradient of *Chaetogaster diaphanus*, however, is unlike that of other naids, and is so peculiar that it would be profitless to describe it. The very young zooids of *Chaetogaster* have, nevertheless, the primary gradient, but as soon as morphological differentiation begins in the zooids, the primary gradient is replaced by the adult gradient, whose chief peculiarity is that the head is least susceptible to cyanide, while a small region at the posterior

end of the stomach is most susceptible. Chaetogaster differs from other naids in a much lower degree of cephalization, and greater differentiation in the alimentary tract, and these differences probably account for its gradient.

In the naids, then, the primary gradient is present only in the zooid stage; in the adult individual there is always present a posterior growing region which has a high rate of metabolism. This region varies in length, but usually includes about the posterior third of the worm. It does not become independent by virtue of its increased rate of metabolism, but continues to be dominated by the cephalic nervous system as shown by the simple fact that it is stimulated on being isolated from the rest of the worm. This stimulation is demonstrable in two ways,—first, by the increased motor activity after isolation, and secondly, by increased susceptibility of such isolated posterior ends to cyanide. It is obvious that an independent part would not be stimulated by physical isolation from the rest of the organism, for it is already physiologically isolated; thus when the head is cut off, it exhibits no increased activity, and no increased susceptibility to cyanide.

With the formation of a zooid in the posterior region, its gradient returns to the primary condition. The head of the zooid becomes dominant over this region; its rate of metabolism grows higher and higher as differentiation proceeds; and with the reorganization of the remaining part to form the new animal, a new independent gradient of the primary kind becomes established.

4. *The gradient of Lumbriculus*

In this oligochaete, the posterior rise in metabolic rate is very marked, owing to the greater length which this worm attains (50 mm. or more). A concentration of $\frac{N}{200}$ to $\frac{N}{400}$ KCN is used for this species. Disintegration begins about simultaneously at both ends, or either end may precede, generally the posterior end. The waves of disintegration then sweep rapidly forward from the posterior end and slowly backward from the anterior end, meeting somewhat anterior to the middle of the worm (fig.

13). The gradient of *Lumbriculus* is not very steep, except at the anterior end, and slight variations, which seem to be related to temporarily increased muscular activity, often occur. Thus if the worm happens to coil itself, the coiled part may show slightly increased susceptibility as compared with surrounding parts, which may be explained as due to contraction of muscles in coiling. Such variations become apparent in a slightly sloping gradient but are concealed in steep gradients such as occur in the naids. The slight slope of the gradient of *Lumbriculus* indicates a low degree of correlation.

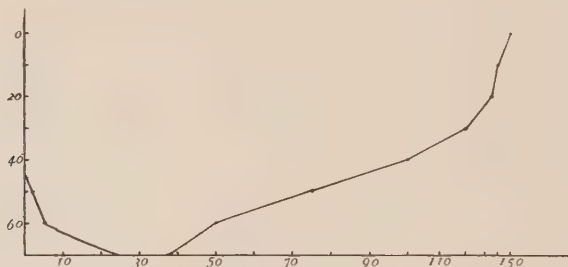


Fig. 13 Graph of the axial gradient of *Lumbriculus inconstans*.

5. *The gradient of the tubificids*

In the tubificids, the posterior rise in metabolic rate has proceeded to a maximum extent. The forms used were *Tubifex tubifex* and *Limnodrilus claparedianus*, and the concentration of cyanide about $\frac{N}{500}$. Disintegration always begins at the posterior end and proceeds far forward before the head begins to disintegrate. The disintegration proceeds very slowly back from the anterior end and meets the posterior disintegration somewhere between the fifth and the fifteenth segments of the body (figs. 14 and 15).

6. *The gradient in embryos*

Before concluding this section, I should like to speak briefly of the gradient of the annelid egg and embryo. Child ('14 a) has worked with the eggs of *Chaetoperus* and *Nereis*, and found

that in the early stages the animal pole shows the highest susceptibility, but that later a second region of high susceptibility appears in the somatic plate from which the three larval segments developed. Among the *microdrilus oligochaetes*, I have been able to obtain young of *Tubifex tubifex* only. In the spring of 1914, nearly all of the individuals of a *Tubifex* culture which had been kept in the laboratory since the preceding autumn became sexually mature and produced a large number of egg capsules. From these capsules, embryos were removed during

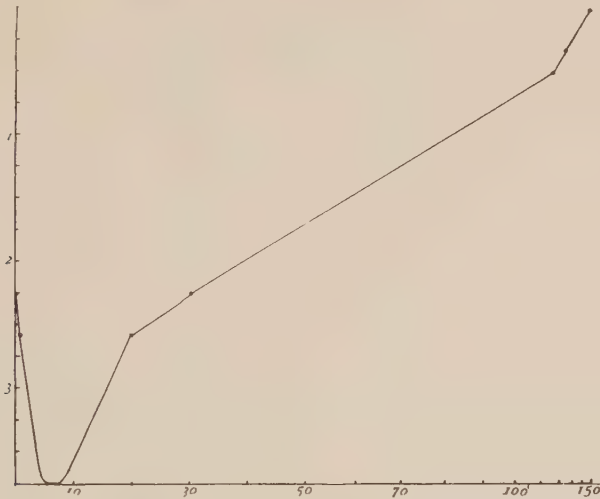


Fig. 14 Graph of the axial gradient of *Tubifex rivulorum*, showing the great extent of the secondary rise. Time in hours.

the later stages of development, and examined in cyanide. It was found that in the stage when the embryo has begun to elongate, its posterior region is most susceptible to cyanide, and the susceptibility decreases anteriorly. Four stages in the disintegration of an embryo at this stage are shown in figure 16. Later, the head becomes a second region of high susceptibility (fig. 17), and the susceptibility of the head increases until it surpasses that of the posterior end. At hatching, therefore, the head is the most susceptible region. The young worms just after hatching consist of about thirty segments. When placed in



Fig. 15 Two stages in the disintegration of *Limnodrilus claparaedianus*.



Fig. 16 Four stages in the disintegration of an embryo of *Tubifex rivulorum*. (The vitelline membrane which surrounds the embryo at this stage is omitted.)

cyanide, disintegration begins at the anterior end, then at the posterior end, and the two waves of disintegration meet at about the middle (fig. 18). As growth proceeds, the susceptibility of the head continually falls, but that of the posterior end remains high because of its unceasing growth which keeps it young. These experiments of Child and myself show that the posterior region of high metabolic rate which is characteristic of the adult annelid arises very early in the embryonic development.

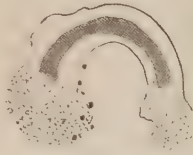


Fig. 17 Stage in the disintegration of a later embryo than figure 16, showing two regions of high susceptibility.

7. *Summary*

These various species of oligochaetes which have been examined form a series, then, with respect to the axial gradient and also, as I shall show later, with regard to regeneration. In *Aeolesoma* the gradient is of the primary type with some indications of a posterior region of high metabolic rate. In the naids, the primary gradient exists in the zooids only, and is modified in the adult by the development of a posterior region of high metabolic rate, which occupies about one-third of the body. In this region the gradient runs in the reverse direction from that of the primary gradient; it may be regarded as a secondary gradient imposed upon the primary gradient as a result of the annelid method of growth. In *Lumbriculus*, the secondary gradient is more extensive than in the naids, including more than the posterior half of the body; and in the tubificids it comprises all of the body except the first few segments.

III. THE DATA OF REGENERATION

1. *The head region of oligochaetes*

I have frequently used the term head region or head in speaking of these organisms and I wish now to explain what is meant by this expression. The head of oligochaetes comprises a

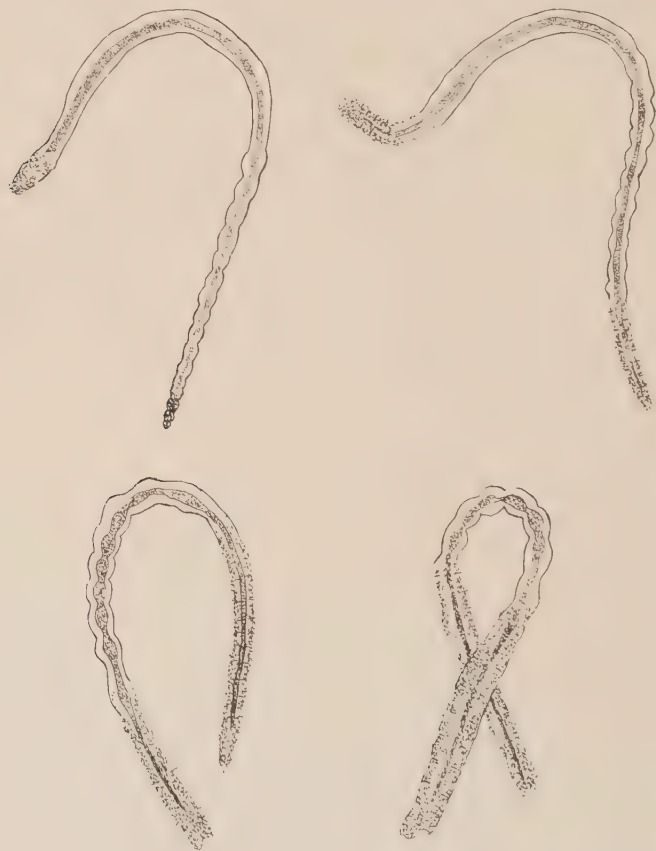


Fig. 18 Four stages in the disintegration of *Tubifex rivulorum* at hatching.

definite number of the most anterior segments, which are generally sharply marked off from the rest of the body; the exact number of segments involved varies with the species. The chief distinguishing characteristic of the head is that it contains the

pharynx, which is completely lacking in chloragogue cells. The outer wall of the remainder of the alimentary tract is composed of these chloragogue cells, which contain numerous brown droplets, apparently metabolic products (since they increase with age), which give to the intestine a characteristic dark brown color. There is thus a sharp line between the white pharynx and the dark intestine, and this line is the boundary between head and trunk. Other less obvious characteristics of the head, which are mentioned by Beddard ('95, p. 11) in his discussion of cephalization in oligochaetes, are: the absence of nephridia, irregularity of the septa, absence of the circum-intestinal loops of the circulatory system, and, in the naids, absence of the dorsal bundles of setae.

That the head of the oligochaetes is a real unit is shown not only by its morphology but by the fact that usually the head segments only are regenerated, no matter how many anterior segments are removed. This statement, although true in general, requires modification, for the regeneration of the anterior end varies according to the size of the piece and the region from which it is taken. Thus *Lumbriculus* regenerates the typical number of head segments only when the piece is of sufficient size. An insufficient investigation of cases such as this has led some investigators to question the existence of a definite head region in oligochaetes. Thus von Wagner ('00) working on *Lumbriculus variegatus* says that the number of anterior segments regenerated in this form is not definite but varies from five to nine. Semper ('76) seems to have been the first investigator to call attention to the sharp differentiation of the head from the trunk in oligochaetes; he observed it in the naids, in which forms it is most obvious. Later Bülow ('83) stated that although there is indeed variation in the number of anterior segments regenerated by *Lumbriculus variegatus*, yet one number—namely nine—predominates. Iwanoff ('03) supports Bülow's conclusion in regard to *Lumbriculus*—although he finds the number to be seven—and has maintained positively that there exists in oligochaetes a head region comprising a fixed number of segments. He uses the same criterion for distinguishing the head as I do, namely, the absence of chloragogue cells.

My own observations on oligochaetes have convinced me that in this group a definite head region is present. It is most sharply differentiated in the naids, where it generally consists of five segments (Dero, Stylaria, Nais). The statement of some authors (Iwanoff, '03) that these forms have but four head segments is probably due to a failure to count the first segment, which, as in all oligochaetes, bears no setae. In *Tubificex tubifex*, there are five head segments; in another species of *Tubifex* which lives in the temporary ponds along with *Lumbriculus* (probably *Tubifex multisetosus*) there are ten head segments. *Limnodrilus* has four, and *Lumbriculus inconstans* seven head segments, although in the latter they are not very sharply defined because the heavy pigmentation of the anterior part of the body conceals the division between head and trunk. In the Lumbricidae, also, there appears to be a definite number of cephalized segments; thus in *Allolobophora foetida* according to the account of Morgan ('97), the number is four or five, and in *A. terrestris* (Hescheler, '98), four. I have been able to find but one exception to this general rule; this is the case of *Criodrilus*, in which form there is apparently no definite cephalization since, according to Tirala ('12), it regenerates up to 28 segments anteriorly, replacing, to a considerable extent, the number removed. However, from the data presented by Tirala, it appears that even in *Criodrilus* there is a tendency to regenerate a certain number of segments, namely, about fifteen, and that with more detailed work on a larger number of individuals this would appear more clearly. I am therefore convinced from my own observations and those of others that the differentiation of a certain number of anterior segments as head segments is practically universal among oligochaetes.

2. General considerations on regeneration in oligochaetes

Regeneration in oligochaetes is always by outgrowth; the head is never replaced within the old tissue, as often occurs in Protozoa, Coelenterates, and flatworms. The process of regeneration, as it occurs in *Dero limosa*, for example, is as follows.

Within a day or so, new tissue begins to grow out from both cut surfaces of the piece. The anterior outgrowth develops faster than the posterior one, but when it has reached a certain length, its growth ceases. Its tip forms a prostomium; mouth, brain and pharynx differentiate within it, and lastly the bundles of setae appear in order, according to the usual law of antero-posterior development. Meantime, the posterior outgrowth has continued its development; within a few days an anus is established, and in front of this anal segment new segments continue to be formed indefinitely but with gradually decreasing rate if no food is given.

Anterior regeneration occurs in a very definite manner in oligochaetes. If the number of segments removed is less than the number of head segments, then only those removed are replaced. If more than the head segments are removed, then the head segments only are regenerated. This fact is not at all remarkable, although it seems to have been so regarded, since it is true of all forms in which regeneration of the anterior end occurs. If pieces are taken from the body of a flatworm, or the stem of a hydroid, the head regenerates directly at the cut anterior surface, and the intermediate parts are not replaced. What really happens is the reorganization of the rest of the body to fit the new head. This reorganization is more extensive in the oligochaetes than in the lower forms, owing to their higher degree of morphological differentiation. The most noticeable changes in these animals are the transformation of the old intestine, which adjoins the regenerated pharynx, into an oesophagus, and the development of the 'hearts' characteristic of the oesophageal region. Histological examination would doubtless reveal many other changes, but I have not attempted to follow the regenerative processes histologically. The normal morphological features of the species are thus restored, even to the formation of the sex organs in parts of the body where they would never have appeared ordinarily (Janda, '12, on *Criodrilus*, and Mrazek, '06, on *Lumbriculus*). Parts anterior to the level of section are never restored unless a head, or an approach to a head is regenerated at the cut surface.

Another general feature of oligochaete regeneration is the inability of the extreme anterior and posterior ends to regenerate. This was noted by Bonnet, and by most investigators since. The head, using the term in its strict sense, when isolated, never regenerates a posterior end; it either dies very soon or else gives rise to 'tailless' heads. The head must have a certain minimum number of body segments behind it before posterior regeneration can occur. This fact, which has long puzzled workers on regeneration, is readily explained on the basis of Child's investigations (a preliminary discussion of these matters appears in the second of the Studies, '11 et. He has pointed out that a large number of data from plants and lower invertebrates show that anterior regions are physiologically dominant over posterior, and the most anterior region, the apical end in plants, and the head in animals, dominates over and controls all other parts of the organism. In regeneration, the first step is the formation of a dominant region, and the development of other parts then follows in correlation with this. For the formation of a dominant part, the cells which are to form it must have a rate of metabolism sufficiently high to enable them to become physiologically isolated and independent of the rest of the piece. If the piece does not contain sufficient material for both dominant and subordinate parts, then the latter cannot arise, as in the case under discussion. This production of dominant parts and nothing else from short pieces is a familiar fact in the case of many coelenterates and flatworms, and occurs also in *Lumbriculus*. Such pieces may also give rise to biaxial dominant structures, if the cells at the posterior end can attain a rate of metabolism sufficiently high to enable them also to become physiologically isolated. Under such conditions, a dominant structure will arise in each of the physiologically isolated regions, namely, at each end of the piece. A piece which does not contain enough material for both dominant and subordinate parts, or which consists to begin with of a dominant part only, can therefore do one of two things,—form a tailless structure, or give rise to biaxial dominant parts.

The posterior end is incapable of regenerating an anterior end unless it be of a certain minimal length. The reason for this is again to be sought in the metabolic relations between the piece and the new cells at its cut surface. The posterior end of oligochaetes, as I have shown, has a high rate of metabolism; unless the new cells at the cut surface can attain a rate of metabolism sufficiently high to enable them to grow at the expense of the old piece, a head cannot form.

Head formation exhibits a progressive decrease along the antero-posterior axis. Posterior regeneration also decreases along the axis; anterior pieces produce more segments posteriorly than do posterior pieces. As the head itself does not regenerate readily posteriorly, as already explained, it is the region behind the head which produces the greatest number of posterior segments. The reason for this appears to be that the more anterior the piece, the higher its level in the primary gradient, and the greater the vigor and intensity of its metabolic processes. As the posterior end develops wholly in correlation with the piece, it follows that the more vigorous the piece, the greater the amount of posterior regeneration, i.e., the more nearly does it approach the normal. Morgulis ('07) has given fully the data regarding regeneration of the posterior end in *Lumbriculus limosus*, and as my own observations regarding number of segments regenerated posteriorly at the different levels of the body agree with his account, I shall not discuss this point any further. On the contrary, the differences in head formation along the axis are of great importance, and will form the subject matter of most of the remainder of this paper.

With these remarks on the general features of regeneration, common to all oligochaetes, I shall now take up the details of regeneration in the four species with which I have worked, *Dero limosa*, *Lumbriculus inconstans*, *Tubifex tubifex*, and *Limnodrilus claperedianus*.

3. *Regeneration in Dero limosa*

While many investigators have stated that the naids possess high capacity for regeneration, yet I have been unable to find any very definite data on the physiological aspects of regeneration in these forms, and no data at all on *Dero limosa*. Most of the regenerative studies on naids have been histological, and have concerned themselves with the germ layer hypothesis, and are, therefore, of little value from the present physiological point of view.

In working on regeneration, I have usually followed the simple method of cutting the worms up into a series of equal pieces along the axis, as fourths, eighths, sixteenths, etc. In this way, one discovers at once the effect of difference in size, and regional differences. A number of worms are cut up at once, and all the pieces of the same level put together. The pieces are kept in large stender dishes or finger bowls, in filtered water, which is changed occasionally. It is not necessary to add any débris except in the case of the naids, where the presence of a small amount of the cultural material seems to reduce the mortality, although it makes the finding of the pieces difficult.

Dero limosa possesses high regenerative capacity. If these worms are cut up into two, three, or four equal pieces along the axis, each piece regenerates a complete anterior and posterior end, with practically no mortality. A complete anterior end is the head of five segments with brain, mouth, prostomium, pharynx, and four sets of setae; a complete posterior end consists of the anal segment with its expanded gill and pavilion, four ciliated gills, and in front of this a variable number of new segments. The new tissue is always readily distinguishable from the old for a considerable length of time, by its lighter color, for it takes some time for the dark granules to accumulate in the chloragogue cells.

With shorter pieces, sixths or eighths—it is not practicable to cut pieces shorter than this in *Dero*,—there is considerable mortality, so that it is difficult to secure constant results. The anterior piece, containing the head, does not regenerate poste-

riorly unless it includes a certain minimum number of body segments. In *Dero*, this number is three, i.e., the anterior piece must be eight segments long before it will regenerate posteriorly. Anterior pieces shorter than this almost invariably die within a few hours after cutting. In only one case did such a piece survive; it consisted of six segments, and did not regenerate posteriorly, forming, in fact, a 'tailless' head. Similarly in short pieces, the last piece, containing the posterior end will not regenerate anteriorly unless it contains 12-15 segments; pieces shorter than this invariably die. The reason for the failure of these anterior and posterior ends to regenerate has been discussed in the preceding section.

As regards the rest of the body, pieces three or four segments long from any part will regenerate complete anterior and posterior ends. Is there then in *Dero* no difference in regenerative capacity along this axis? An axial difference does exist and reveals itself in the following ways; anterior pieces regenerate faster than posterior ones; the regenerated head is larger on anterior pieces than on posterior pieces; the number of segments regenerated posteriorly is greater in pieces from the anterior regions of the body, and decreases along the axis; smaller pieces from the anterior regions will regenerate completely than from the posterior regions. In regard to this last point, pieces from the anterior half of the body, containing only one or two segments will regenerate into complete worms; while from the posterior half of the body, pieces must be at least three or four segments long before this can occur. These facts indicate an axial difference, which is, however, relatively slight as compared with other forms.

Biaxial heads arise in short pieces of *Dero*; two such were observed, and undoubtedly more could be readily produced. One of the observed pieces consisted of about three segments; it regenerated a complete head at each end (fig. 19). The other piece consisted of little more than one segment; in this case, the anterior head was complete, but the posterior one regenerated only three setigerous segments, instead of the normal four. This piece was cut in two, but both portions died. An adequate

explanation of the formation of biaxial heads has been given by Child ('13 b). The original gradient must be largely eliminated, and this is accomplished by cutting short pieces,—which include such a small portion of the original gradient that there will be little difference in rate of metabolism between the two ends of the piece,—or by depressing the rate of metabolism of the pieces (low temperature, cyanide, anaesthetics, etc.). If, then, the growing cells at the two cut surfaces of these pieces in which the gradient has been eliminated attain a sufficiently high rate of metabolism, each will develop an independent part, that is to say, a head. If, on the contrary, the rate of metabolism of the old piece remains higher than that of the growing cells, it will dominate over them, and they will be able to give rise to subordinate structures only, namely, tails. Child has been able to



Fig. 19 Biaxial heads in *Dero limosa*.

demonstrate the correctness of this explanation as follows. *Planaria dorotocephala* seldom gives rise to biaxial heads, but if short pieces are put into depressing agents to eliminate the gradient, and then allowed to regenerate in water, a considerable percentage of biaxial heads is obtained. As far as I am aware, biaxial heads have not previously been observed in annelids although a reversal of polarity of a similar character has been produced in lumbricids by grafting short pieces in the reverse position upon the anterior end of long pieces (Hazen, '99 and Rutloff, '08).

Regeneration in *Dero* is nearly always complete and 'normal.' In anterior regeneration, the only variation from the normal is the occasional appearance of heads containing fewer or more than the five typical head segments. Such heads may be designated as hypomeric, when the number of regenerated segments is less than normal, or hypermeric, when it is more. Hypo-

meric heads, consisting of only four segments, of which three are setigerous, but normal in all other respects, occur occasionally; but heads containing less than four segments have never been observed. Hypermeric heads are rare, but I have observed a few, consisting of six, and, in one case, seven segments; in such heads, the setae tend to be irregularly disposed, so that one is uncertain of the boundaries of the segments. The hypomeric and hypermeric heads do not regulate to normal, at least in the time which I observed them, nor is the abnormal number of head segments inherited in asexual reproduction. In posterior regeneration, there arise, not infrequently, abnormalities in the shape of the gill pavilion, and in the size, and number of the gills—one, two, or three appearing instead of the normal four; but in the case of posterior structures, regulation to normal usually occurs.

In regard to the regeneration of *Dero*, then, the important fact is that complete anterior regeneration occurs everywhere along the axis (with the exception of the extreme posterior end).

4. *Regeneration in Lumbriculus inconstans*

Lumbriculus inconstans is undoubtedly the most favorable oligochaete for experimental work on account of its large size, high capacity for regeneration, low mortality, and, most important of all, because it exhibits *qualitative* axial differences.

It has this disadvantage, however, that, owing to its peculiar habitat, it can be collected only in the spring months; freshly collected material is necessary for experimental work because the vitality of the worms decreases when they are kept for any length of time in the laboratory. Owing, therefore, to the relatively short time during which material is available, my experiments on this most instructive species are as yet incomplete in many details.

The genus *Lumbriculus* has long been noted for its high regenerative capacity, and the species *L. inconstans* is no less remarkable in this respect than are its relatives, *L. variegatus*, a favorite object of research with European investigators, and

L. limosus, on which Morgulis ('07) has experimented. *Lumbriculus inconstans*, however, differs from both of these forms in a very important way as will appear shortly.

If *Lumbriculus inconstans* is cut up along the axis into pieces of moderate length,—that is, pieces including more than ten segments—then each of these regenerates completely. A complete anterior end is the head of seven segments, of which six are setigerous, a large prostomium, brain, pharynx, etc.; but the transverse green pigment stripes do not appear on the regenerated head for some time. In long pieces, the regeneration of seven head segments is almost invariable, demonstrating that a definite number of segments is differentiated into a 'head' in *Lumbriculus*; but in short pieces, the number of head segments regenerated is very variable, and this variability has led to much controversy over the question of cephalization in the oligochaetes. The regeneration of the posterior end consists simply of the formation of an anal segment, and of an indefinite number of new segments in front of this. As is general in oligochaetes, neither the extreme anterior or posterior ends are capable of regeneration. The head piece must include at least four trunk segments, or eleven in all, before it will regenerate posteriorly; isolated heads shorter than this remain tailless. The posterior end must be twenty or thirty segments long before it will regenerate anteriorly, otherwise it dies without regeneration.

With shorter pieces, less than ten segments long, an axial difference becomes apparent. Disregarding now the extreme anterior and posterior piece, it is found that the capacity for both anterior and posterior regeneration decreases along the axis. Posterior regeneration may be dismissed briefly. Pieces from the anterior regions, although they may remain tailless in a small percent of cases, in which the pieces are too short to contain enough of the axial gradient for formation of both dominant and subordinate parts (see above, and Child, '13 b), in general produce very long posterior ends. As one passes back along the axis, the number of posterior segments regenerated gradually decreases, and in pieces from posterior regions, frequently only

the anal segment is regenerated, although such pieces are never tailless. With the exception, then, that extreme anterior pieces may remain tailless, all levels of the Lumbriculus body regenerate normal posterior ends, but the amount regenerated decreases along the axis.

In anterior regeneration, the character of the anterior outgrowth depends upon the level of section, and shows all gradations from a normal head to a normal tail. In an axial series of short pieces, the most anterior ones regenerate normal heads, usually of seven segments, but not infrequently hypo- or hypermeric. Pieces farther back tend to produce structures which depart more and more from the normal head; and pieces from posterior regions give rise to various types of abnormal, or, as I prefer to call them, inhibited structures, among which are represented all gradations from a normal head to a normal tail. I have attempted to classify these various kinds of anterior structures into several categories, although it must be understood that they grade into each other perfectly. The following description together with the accompanying figures will give some idea of their appearance.

1. Normal heads. This head has a large well-developed prostomium, mouth, pharynx, supraoesophageal ganglia with circumoesophageal connectives, and usually consists of seven segments, of which six are setigerous (fig. 20 b). It resembles in all respects the head of the animal as it occurs in nature (fig. 20 a), except that the transverse pigment stripes are lacking on the regenerated head. The typical number of segments in the normal head is seven, but in short pieces, the number of regenerated segments varies from four to nine. Normal heads are regenerated at any level of the Lumbriculus body, but in short pieces, the frequency of their occurrence decreases, and the tendency to hypomerism increases along the antero-posterior axis.

2. Hypoprostomic heads. These differ from the normal in that the prostomium is reduced in size, and frequently abnormal in shape (fig. 20 c). The hypoprostomic head has a brain (which has not been investigated histologically) mouth, and pharynx,

but is usually hypomeric. The percentage of such heads is small in pieces from anterior regions, but increases along the axis.

3. *Aprostomic heads*. This type of inhibited head consists of a rounded outgrowth, in which the prostomium is entirely

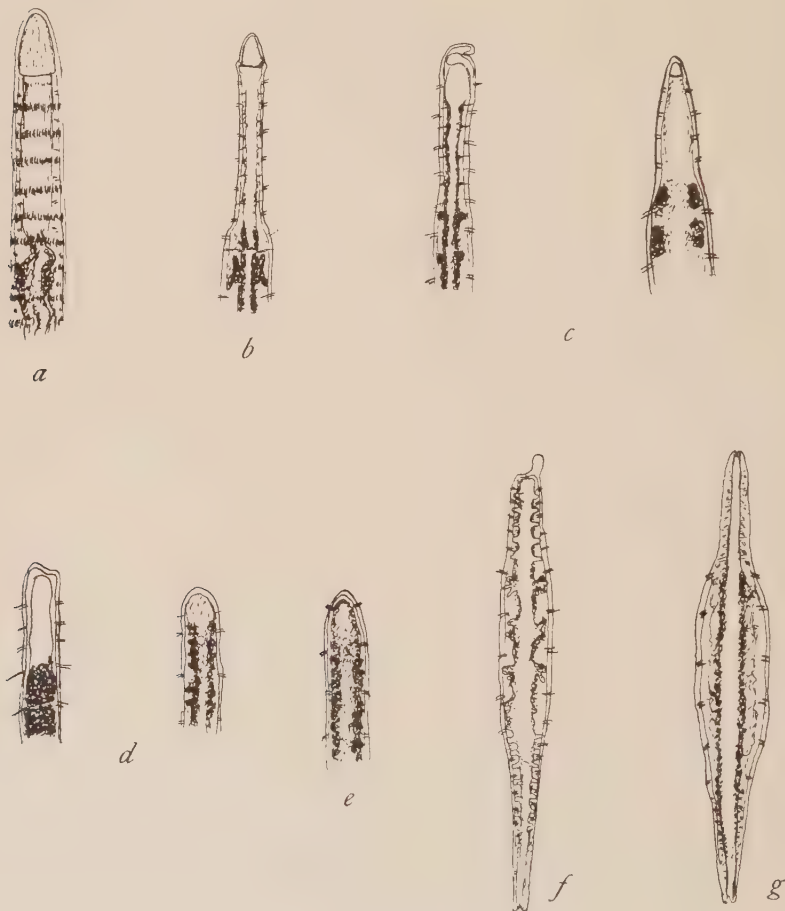


Fig. 20 Types of anterior outgrowths in *Lumbriculus*. *a*, normal head in nature; *b*, normal regenerated head; *c*, two types of hypoprostatic heads; *d*, two aprostomic heads, which are also markedly hypomeric; *e*, an acephalic condition; *f*, cephaluran outgrowth; *g*, biaxial tails.

lacking. (fig. 20 d). There is no mouth, the pharynx ending blindly. Histological examination shows that the supraoesoph-

ageal ganglia, and circumoesophageal commissures are absent, and that the ventral nerve cord terminates in a mass lying beneath or in front of the blind end of the pharynx. It seems very probable to me that the brain and commissures do not develop as distinct structures because the mouth is absent. The aprostomic head is always hypomeric, usually markedly so, and like the preceding type is of most frequent occurrence in pieces from posterior regions.

4. *Acephalic pieces.* In these pieces, the cut end simply heals over and no outgrowth takes place (fig. 20 e). These headless pieces are rare in *Lumbriculus*, and are confined to posterior levels of the body.

In these four classes of structures, we have, as in *Planaria dorotocephala* (Child '11 b) all gradations from the normal head to the acephalic condition. There is a gradual reduction of certain cephalic structures, the prostomium, cephalic nervous system, mouth, and number of head segments, ending with complete inhibition of head formation. The various types of heads, are not, however, so definitely localized along the axis, as is the case in *Planaria*, although in general, the percentage of normal heads decreases along the axis, and the percentage of inhibited heads increases. *Lumbriculus* further differs from *Planaria* in that in place of acephalic pieces, posterior structures may arise. I have distinguished two kinds of these.

5. *Cephaluran outgrowths.* This interesting type of anterior outgrowth appears to have, as the name adopted for it implies, characteristics of both head and tail (fig. 20 f). In my opinion, such an outgrowth starts as an inhibited head, an aprostomic head, but is unable to maintain sufficient dominance over the old piece to continue to develop as a head; its further development is therefore that of a subordinate part, a tail. The cephaluran outgrowths have a rounded or pointed end in which there is a terminal nerve mass like that found in the aprostomic heads: like the latter, also, there is no opening to the exterior, neither mouth nor anus. The tail characteristics of these structures are the following: large number of segments, for they are nearly always hypermeric, decrease in the size of the segments ante-

riorly, presence of a pair of transverse blood vessels in each segment, and, in some cases, reversal of the direction of blood flow, such as occurs in the biaxial tails. Tail characteristics predominate but the cephaluran structure differs from a tail in the absence of the anus, and the presence of the nerve mass. Cephaluran outgrowths arise in pieces from posterior levels only.

6. *Biaxial tails.* The regeneration of tails at both ends of pieces has long been known in *Lumbriculus*; like the preceding type of structure, biaxial tails arise in pieces from posterior regions only. Both tails are identical in structure, the anterior tail being however usually shorter, (fig. 20 g); and the blood always flows from the tip of each towards the middle of the piece.

7. *Multiple outgrowths.* In this category I have grouped all those cases in which multiple structures arise at the cut surface. Such multiplication of parts is limited to anterior regeneration. The commonest case of this kind is the duplication of the head; and all gradations are found from two completely separate heads to cases where only the prostomia are separate, as in figure 21. The double structure may consist, however, not of two heads, but of head and a tail (fig. 22); this is not a case of axial heteromorphosis, for here the tail grows out in correlation with the new head, while the biaxial tail develops in correlation with the old piece. If both parts of a double outgrowth develop with equal rates of metabolism, then neither one will be able to control the other, and both will give rise to heads; but if one of the outgrowths attains higher metabolic activity than the other, it will become a dominant part, a head, compelling the other outgrowth to develop as a subordinate part, a tail. The result is a head with two tails. Pieces of *Lumbriculus* may give rise to three or four such outgrowths at their anterior ends, some of which are heads of various kinds, and some of which are tails.

To illustrate now the distribution of these different types of anterior outgrowth along the axis, the results of a series of twenty-five worms cut up into sixteenths are given in table 1. As the first sixteenth pieces include the original head, and the last sixteenth pieces all died, they are omitted from the table.

This table shows that the different kinds of anterior structures are not very sharply localized along the axis, but that in general, the percentage of inhibited forms is greater in posterior regions. The increase in percentage of normal heads in extreme posterior pieces (fourteenth and fifteenth pieces in the table) appears to be a general result, and is probably due to two factors: first, owing to the decrease in size of segments posteriorly, these pieces

TABLE 1

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Normal.....	21	17	15	18	17	16	18	14	15	7	4	6	11	14
Hypoprostomic.....	3	4	3	4	1	2	2	1	3	3	6	5	1	1
Aprostomic.....		2	3	3	5	3	2	1	3	7	9	6	7	1
Acephalic.....												2		
Cephaluran outgrowths.....									1	2	4	2	2	
Biaxial tails.....										1	1	2		
Multiple outgrowths.....										3	1		1	
Dead.....	1	2	4	0	2	4	3	9	3	2	0	2	3	9

TABLE 2

										1	1		1	
9 segments.....										2		1	2	
8.....	1		2								1	2		
7.....	21	16	11	16	18	11	18	12	14	7	4	2	1	1
6.....	2	6	5	4	2	7	3	3	3	4	5	5	5	3
5.....			1	4	2			2	2	4	7	2	8	3
4.....		1	2	1	2	3	1			1	1	3	2	6
3.....											1		1	1

contain more segments than anterior pieces of equal length, and, secondly, these pieces are in the region where autotomy occurs,⁴ and where, therefore, new individuals already exist to some extent physiologically. Similarly, in *Planaria dorotocephala* (Child, '11 b), pieces from the levels where the zooids are situated generally produce normal wholes.

In table 2, the number of head segments regenerated by the same sixteenth pieces is given (cephaluran outgrowths and biaxial

⁴ *Lumbriculus inconstans* reproduces asexually by autotomy. Upon stimulation, this worm breaks in two at a rather definite level, near the posterior end of the body.

tails being omitted). It is seen that anterior pieces usually regenerate seven head segments, while posterior pieces show a marked hypomerism.

The most striking fact about the regeneration of *Lumbriculus inconstans* is, then, that there exists an axial difference in head



21



22

Fig. 21 Multiple outgrowths; double prostomia.

Fig. 22 Multiple outgrowths; head and tail.

formation. In short pieces normal heads tend to be formed from anterior levels, but as one passes posteriorly along the axis, the process of head formation is inhibited to a continually increasing degree.

5. Regeneration in the tubificids

In these forms, the inhibition of head formation has proceeded to such an extent that all pieces back of a certain anterior level remain headless. By removing definite numbers of anterior segments, one may determine the level at which head formation ceases. In the case of *Tubifex*, five, ten, fifteen, and twenty anterior segments were removed, with the following results. At the level of the fifth segment, a normal head is usually regenerated. The normal head of *Tubifex* consists of five segments, four setigerous, and contains mouth, brain, pharynx, etc. When ten anterior segments are removed, the result is the same. When fifteen are cut off, some normal heads are regenerated, but most of the pieces give rise to inhibited heads, and some remain headless. I find in my notes unfortunately, no description of the appearance of these inhibited heads, nor any statement as to whether various types could be distinguished. At the level of the twentieth segment, all pieces remain headless. Between the fifteenth and the twentieth segments, therefore, head formation ceases rather abruptly, although inhibited heads occur as stages between the normal and the acephalic condition. These results agree with those of European investigators working on the same species, *Tubifex tubifex*, except that they found head formation to cease at a more anterior level—tenth to twelfth segment (Abel '02, Haase '98). The regeneration of the head is slow in *Tubifex*, and the greater the number of anterior segments removed, the more slowly does regeneration take place.

In *Limnodrilus claperedianus*, the capacity for head formation is even more limited than in *Tubifex*. As this species has already been thoroughly investigated by Kreeker ('10), it was not necessary for me to perform any very extensive experiments. The head of *Limnodrilus* consists of four segments, but two is the maximum number regenerated; of these, the first bears prostomium, mouth and brain, all of normal appearance, and the second is setigerous. From this condition, one finds the following gradations to the acephalic state; regeneration of one segment only with normal prostomium, mouth, and brain, reduction of

prostomium, aprostomic condition ('rounded ends' of Kreckler), and finally the acephalic condition, where no tissue grows out at all. All pieces from which more than seven anterior segments are removed remain acephalic.

As regards posterior regeneration, all levels of the body regenerate normal posterior ends, with the usual exception that the head itself remains tailless unless it includes a certain minimum number of body segments.

6. *Summary*

It thus appears from these data that the oligochaetes investigated form a series with regard to head formation. In *Dero*, normal heads are formed at all levels of the body, in both short and long pieces; in *Lumbriculus*, normal heads occur at all levels in long pieces, but in short pieces, head formation exhibits progressive inhibition along the axis; and in the tubificids, head formation is limited to extreme anterior levels, regardless of size of piece. It seems to me that the earthworm and its relatives occupy an intermediate position between *Tubifex* and *Lumbriculus*. Thus in *Allolobophora foetida* described by Morgan ('97), regeneration of the head occurs with increasing difficulty back to the level of the twelfth to fifteenth segments; behind this level is a region where pieces either remain headless or produce bi-axial tails, and posterior to this all pieces remain headless. In the tubificids, head formation ceases at anterior levels without any intermediate production of biaxial tails.

The regeneration of the anterior end exhibits, then, these striking axial differences which are of a qualitative nature. On the other hand, the regeneration of the posterior end occurs at all levels of the body in all of these oligochaetes, and with all sizes of pieces (with the exception of the head piece, which remains tailless unless of a certain size). The axial differences in posterior regeneration are quantitative merely.

IV. ANALYSIS OF THE REGENERATIVE PROCESS

A consideration of the data presented above leads us to the conclusion that the formation of an anterior end is a different process from the formation of a posterior end. The fact that in a set of pieces of *Lumbriculus* of equal size, and taken from the same level of the body, any type of anterior outgrowth from a normal head to a normal tail may be obtained, proves that the kind of anterior structure which is to arise is not predetermined in the piece, but is due to dynamic conditions arising within the piece after its isolation from the whole. The previous position of the piece as part of the whole is only an indirect factor in head determination.

The consideration of a similar set of facts obtained from his experiments with *Planaria dorotocephala* led Child ('13 b, '14 e) to an analysis of anterior regeneration in this animal in metabolic terms. In the following pages a similar analysis is made for *Lumbriculus inconstans*.

1. The time of head determination

It is necessary to know, as the first step towards the understanding of the process of anterior regeneration, at what time the head is determined in a regenerating piece. This can be readily discovered in the case of organisms which show progressive inhibition of head formation along the axis by means of the method devised by Child ('14 e). The piece *ac* in figure 23 gives rise to normal heads in 90 to 95 per cent of cases (the remaining forming mostly multiple structures). The piece *ab* with the same anterior level will, however, produce under ordinary conditions only 20 to 30 per cent of normal heads, and 70 to 80 per cent of inhibited heads and other types of anterior outgrowths. It is therefore obvious that whether or not the cells shall give rise to a normal head is dependent upon their forming part of the long piece *ac*; and if they remain a part of *ac* for a sufficient length of time, then they should, when isolated, give rise to nothing but normal heads. To discover the period of time required for the determination of the head as normal, it is only necessary

to cut a large number of pieces *ac*, and at different intervals after this operation to cut off pieces *ab* from the anterior ends of



Fig. 23 Semi-diagrammatic figure of *Lumbriculus inconstans*, to show levels of section.

the *ac* pieces. These *ab* pieces are then allowed to regenerate, and it is found that the percentage of normal heads produced

by them runs parallel to the length of time that they were allowed to remain part of the long pieces.

Experiments of this kind carried out on *Planaria dorotocephala* (Child, '14 e) yielded the unexpected result that it is determined within six to eight hours after section whether or not a piece of *Planaria* shall produce a head or remain headless, and within eighteen hours whether the head shall be normal. In the case of *Lumbriculus*, acephalic pieces are so rare that one cannot discover the time at which head determination occurs; I have therefore merely attempted to find out when the head is determined as normal. As preliminary experiments showed that at least ten hours are required for this, I began my later experiments with a fifteen hour interval. The result of such an experiment are given in table 3. Two hundred long pieces *ac* were cut from

TABLE 3

TIME BETWEEN TWO OPERATIONS	NORMAL	HYPOPROSTOMIC	APROSTOMIC	BIAXIAL TAILS	DEAD
No hours.....	20	42	12		26
15 hours.....	60	30	6	2	2
20 hours.....	70	26	2		4
30 hours.....	88	4			8

the posterior ends of worms of the same size and from the same stock; fifty short pieces *ab* were cut from the anterior ends of these immediately; and fifty more after elapse of fifteen, twenty, and thirty hours respectively. The results are given in percentages; multiple outgrowths are classified under the types of heads. While I regret that I have not a closer series of time intervals, yet I think that it is evident that the head of *Lumbriculus* is determined as normal in the majority of cases within twenty hours after section, and in practically all cases within thirty hours.

2. Stimulation by section

As a second step in the analysis of the process of anterior regeneration, one must know what the metabolic condition of the pieces is at the time when the head is determined as normal.

The rate of metabolism of pieces is not the same as that of corresponding parts of the intact worm because, as Child has shown ('14 d), the rate of metabolism is increased by the operation of cutting; this increase is greater the shorter the piece, and the lower its previous rate as part of the axial gradient. These facts have been demonstrated not only by the increased susceptibility of the pieces to cyanide, but also by their increased CO_2 production in Tashiro's biometer. Similarly, in all the oligochaetes which I have tested *Dero*, *Lumbriculus*, *Tubifex*, and *Limnodrilus*—stimulation results from section. In long pieces there is little stimulation, and the disintegration gradient of the intact worm is preserved in the pieces, except for increased susceptibility at the cut surfaces. Short pieces are stimulated to a much greater extent, and their disintegration in cyanide takes place without regard to the previous gradient; it begins at the cut surfaces and proceeds towards the middle. It is therefore the region of the wound which is the seat of the stimulation, and in short pieces the wound regions practically include all of the piece. Stimulation as a result of injury is undoubtedly a general phenomenon exhibited by living matter (Tashiro, '13), and gives us a simple explanation of such facts as the current of injury of nerve and muscle.

The degree of stimulation of pieces is a function of their previous axial position, and this is particularly noticeable in short pieces. The isolated head shows no stimulation; pieces from anterior regions where the metabolic rate is high are stimulated to some extent; pieces from middle regions where the rate is lowest are stimulated most; pieces from the posterior region of high rate are stimulated to a considerable extent, and since they already possessed a high rate before section, their rate after section is usually higher than that of the middle pieces, although the rate of the latter has been increased more relative to their previous rate. The metabolic condition of the axial series of pieces is not, therefore, the reverse of the metabolic gradient of the intact animal, as is the case in *Planaria*, because of the existence in oligochaetes of the posterior region of high rate. That this posterior region is stimulated by section indicates, as I have al-

ready pointed out, that it is not a truly independent part. The reversal of the gradient after section appears most clearly in a form like *Dero*, where the posterior region is of small extent. Table 4 is the record of such an experiment on *Dero limosa*; ten worms were cut up into sixth pieces, and put immediately into $\frac{N}{500}$ KCN. The experiment was begun at 12.45, and the number of pieces completely disintegrated recorded at fifteen minute intervals.

In general, then, the regions which in the intact animal have lowest susceptibility have highest susceptibility after section, while the regions having high susceptibility in the whole are not much altered by section, with the exception of the posterior end

TABLE 4

TIME	1	2	3	4	5	6
1.00.....	0	0	0	0	0	0
1.15.....	0	1	2	2	2	1
1.30.....	0	4	5	3	4	2
1.45.....	0	7	8	5	4	6
2.00.....	0	8	8	8	6	8
2.15.....	1	8	8	9	7	9
2.30.....	4	9	10	9	7	9
2.45.....	10	10		9	8	10
3.00.....				10	9	
3.15.....					10	

which although already having a high rate is somewhat simulated by section. In the case of *Lumbriculus* and the tubificids, where over half of the body is involved in the posterior rise of metabolic rate, the susceptibility of all pieces except the most anterior is increased by section, that of the middle pieces relatively most but not enough to raise it above that of posterior pieces which had a much higher rate before section. The gradient is therefore not reversed in these forms; it simply is raised to a higher level, and becomes less steep.

The stimulation following section may be explained as due to the severance of conduction and correlation paths. The fact that the head and anterior regions in general are little stimulated by isolation indicates that they are relatively independent of

other parts; posterior regions are, on the other hand, dependent on anterior parts and subordinate to them, since the severance of conduction paths between them results in stimulation.

The increase of metabolism after section is only temporary. If the pieces are tested in cyanide at various intervals after cutting, it is found that the susceptibility to cyanide gradually decreases to far below normal, and then begins to rise again as regeneration sets in. For an analysis of the process of head formation, it is necessary to know what the metabolic condition

TABLE 5

Susceptibility of short anterior piece de to KCN $\frac{N}{500}$ at various times after cutting

TIME	SAME REGION OF CONTROL	IMMEDIATE- LY AFTER SECTION	5 HRS.	17 HRS.	24 HRS.	48 HRS.	4 DAYS
1.45.....	0	0	0	0	0	0	0
2.00.....	0	1	1	0	0	0	0
2.15.....	0	2	1	0	0	0	0
2.30.....	0	5	1	0	0	0	0
2.45.....	1	8	2	0	0	0	0
3.00.....	4	10	3	0	0	1	0
3.15.....	8		3	0	0	1	1
3.30.....	9		7	0	1	1	3
3.45.....	9		7	0	1	1	3
4.00.....	9		9	0	3	4	8
4.15.....	10		9	1	4	6	10
4.30.....			10	4	6	7	
4.45.....				6	7	10	
5.00.....				9	9		
5.15.....				9	9		
5.30.....				10	10		

of the pieces is during the period in which the head is determined. I have therefore tested the susceptibility to cyanide of short anterior and posterior pieces of *Lumbriculus*. In table 5 is given the susceptibility to cyanide of the anterior piece *de* in figure 23, immediately, 5, 17, 24, 48, and 96 hours after section; and in table 6, the susceptibility of the posterior piece *ab* in figure 23 immediately, 5, 14, 19, 24, 48, and 96 hours after cutting. Ten pieces are used in each case, and the time of death of corresponding regions of whole worms also noted. The concentration of cyanide used was $\frac{N}{500}$; observations were taken every

fifteen minutes, and the number of pieces completely disintegrated recorded.

The general result of experiments of this kind is that the rate of metabolism of anterior pieces falls rapidly after cutting, and rises again, so that within four days, it has reached the normal rate. In posterior pieces, the rate stays up longer after cutting, then falls to a greater extent, and remains low for a longer period of time. Further experiments show that it begins to rise by the

TABLE 6

Susceptibility of short posterior piece ab to KCN $\frac{N}{500}$ at various times after section

TIME	SAME REGION OF CONTROL	IMMEDI- ATELY AFTER SECTION	5 HRS.	14 HRS.	19 HRS.	24 HRS.	48 HRS.	4 DAYS
1.45.....	0	0	0	0	0	0	0	0
2.00.....	0	0	1	0	0	0	0	0
2.15.....	0	1	1	0	0	0	0	0
2.30.....	0	4	2	1	0	0	0	0
2.45.....	0	5	3	1	0	0	0	0
3.00.....	0	7	4	2	0	0	0	0
3.15.....	0	9	6	4	2	1	1	0
3.30.....	1	9	7	7	4	3	1	0
3.45.....	3	10	9	8	5	3	1	0
4.00.....	4		9	10	6	3	1	0
4.15.....	5		9		6	4	1	0
4.30.....	10		10		7	5	3	1
4.45.....					9	6	5	1
5.00.....					10	7	7	3
5.15.....						8	8	4
5.30.....						10	10	4
5.45.....								6
6.00.....								7

sixth day. Eventually the rate of metabolism of all regenerating pieces becomes much higher than their original rate as parts of the organism; rejuvenation thus occurs as a result of regeneration.

The rate of metabolism, then, of anterior pieces of *Lumbriculus* is low during the period that the head is determined as normal; and these pieces give rise to a high percentage of normal heads. On the contrary, the rate of metabolism of posterior pieces is

high during the time of head determination; and it is these pieces which produce inhibited structures.

3. General conception of the process of regeneration

This relation between frequency of normal head formation, and rate of metabolism at the critical period of head determination in *Lumbriculus* has lead me to accept the conclusions to which Child has come from the consideration of similar facts in *Planaria dorotocephala*. Since he has already presented his conclusions (Child, '14e), it is not necessary for me to discuss them in any very great detail. For the following brief presentation, it will be convenient to employ a figure similar to that frequently used by Child (fig. 24). The cells at the cut surface x

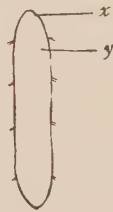


Fig. 24 Diagram for theory of head formation.

as a result of the wound and altered conditions begin to produce new tissue from which the new head is to arise. These cells grow out with a certain rate of metabolism which is relatively high as shown by disintegration experiments. Now if the rate of metabolism of the old tissue y is low, there is nothing to hinder x from continuing its development; it becomes dominant over y , uses up the material of y for regeneration, and produces a normal head. This is the case in anterior pieces of *Lumbriculus* where, as was shown in the preceding section, the rate of y is low during the time that the head is determined as normal. If, on the contrary, the rate of y is high, then x will not be able to dominate over y , nor to attain sufficient independence to produce a normal head; the development of x will be inhibited, and in proportion to the metabolism of y . Thus are produced the various types of inhib-

ited heads; the higher the rate of y the more will it inhibit the anterior structure which arises, and if the rate of y is sufficiently high, then region x will not be able to become independent at all but will be dominated by y , and give rise to a subordinate part, a tail. Under such conditions biaxial tails result. It is also conceivable that x might begin head development but later be dominated by y to such an extent that head formation can no longer continue but tail formation sets in; this in my opinion is the explanation of the cephaluran outgrowths. In the case of multiple outgrowths, the outgrowth or outgrowths which have the highest rate become heads, and dominate over the others which then give rise to tails. In *Planaria*, when the region y dominates the region x , the acephalic condition results; but this is not the case in *Lumbriculus*, probably because the new tissue begins to grow out before its fate has been decided, and it then forms a head or a tail according as x or y dominate.

It is not necessary that the region y should have a metabolic rate which is actually higher than that of x ; in fact it probably never has, for disintegration experiments show that x usually disintegrates before y . But it is the ratio of the rate of x to the rate of y which is important, and in all probability the rate of x must exceed the rate of y by a considerable amount before normal head formation can occur. Relatively slight alterations of the ratio $\frac{\text{rate of } x}{\text{rate of } y}$ are sufficient to affect the process of morphogenesis. Thus is a set of pieces of the same size, and from the same level of the body, the value of y cannot be very different in the different individual pieces; the rate of x probably differs even less; yet from such a set of pieces all types of anterior structures are obtained.

If this conception of the process of head formation is correct, an experimental control of morphogenesis should be possible. By depressing the rate of y as compared with x , one ought to be able to increase the percentage of normal heads; and, conversely, by increasing the rate of y as compared with x , a decrease in the percentage of normal heads should result. The first possibility is readily realized experimentally; the rate of y can be easily

lowered by placing the pieces in dilute cyanide solutions immediately after section. While this treatment must depress the rate of x also, yet the depression is less than in the case of y , because x is young, growing tissue, and regulates better to the depressing condition. The desired increase in the value of the ratio $\frac{\text{rate of } x}{\text{rate of } y}$ is thus attained, and as postulated, the percentage of normal heads is decidedly increased. This beautiful experiment was first performed by Child on *Planaria dorotocephala*, and is readily repeated in the case of *Lumbriculus inconstans*. In Table 7 are given the results of such an experiment. One hundred short posterior pieces (ab in fig. 23) were cut; fifty were put into water as control and fifty put immediately into cyanide solution of concentration $\frac{N}{25,000}$ for four days, then removed to water.

TABLE 7

	NORMAL	HYPOPROS- TOMIC	APROSTOMIC	ACEPHALIC	BIAXIAL TAIL	DEAD
Control.... .	18	30	24	6		22
Experiment....	46	16	22		2	14

As the ab pieces never in my experience yield more than 30 per cent of normal heads, the increase here is due to the depressing action of the cyanide.

The converse experiment, that of decreasing the ratio $\frac{\text{rate of } x}{\text{rate of } y}$ is very difficult to carry out, for it is almost impossible to increase the rate of y , without at the same time increasing the rate of x more. Therefore stimulating conditions, such as increased temperature, increased motor activity produce the same effect as in the preceding experiment, as Child has shown; the rate of x is increased more than that of y , and a higher percentage of normal heads results. In *Lumbriculus inconstans*, however, the desired condition is realized in an unexpected way; in my earliest experiments upon this species I soon found that the 'vitality' of the worms decreases greatly if they are kept for any length of time in the laboratory. The capacity for regeneration diminishes to such an extent that the worms can no longer be used for

experimental purposes. The cause of the decrease in regenerative power appears to be that the cells at the cut surface fail to grow out with their usual vigor. The rate of x is thus lowered, as required in the experiment, and the expected decrease in the percentage of normal heads occurs. In table 8, a comparison is made between the regenerative capacity of *ab* pieces cut on April 24, and two lots cut on May 30, from the same stock. Fifty pieces were taken in each case, and the results are expressed in percentages.

TABLE 8

TIME OF CUTTING	NORMAL	HYPOPROS- TOMIC	APROSTOMIC	ACEPHALIC	BIAXIAL TAILS	DEAD
April 24.....	18	30	24	6		22
May 30.....	6	34	20	2	6	32
May 30.....	4	20	28	6	6	36

The decrease in normal heads is very characteristic, also the appearance of biaxial tails. The number of normal heads may be considerably increased in these pieces by putting them in dilute cyanide. The mortality in these pieces cut late in the season is unfortunately always high, but as the decrease in normal heads has been noticed in every experiment performed with worms kept some time in the laboratory, it cannot be accounted for on the basis of the increased mortality.

The rôle of the axial gradient in morphogenesis has not perhaps been sufficiently emphasized. In the case of short pieces, the dynamic conditions developed after section are the principal factors, yet it should be obvious from what has been said that these conditions are dependent on the previous position occupied by the pieces in the axial gradient of the intact animal. In long pieces, the axial gradient is more directly concerned; these pieces are only slightly stimulated by section, and the axial gradient is preserved in them in practically its original state. Therefore the anterior end of long pieces always has the highest rate in the piece, and the region x is never threatened with inhibition from the parts behind it. For this reason, long pieces of *Planaria* and *Lumbriculus* always give rise to normal heads. It is the

axial gradient also which determines that the head shall arise at the anterior end of the piece (Child, '14 e, p. 73).

The objection may be raised to these statements that in some of the oligochaetes which I have been considering, the axial gradient is such as to be higher at the posterior end than at the anterior end of long posterior pieces. Now there appears to be a correlation between the extent of the characteristic posterior rise in metabolic rate, and the capacity for head formation. Thus in *Dero*, where the posterior rise is of small extent, head formation occurs at all levels; in *Lumbriculus*, where the region of high rate occupies more than the posterior half of the body, head formation tends to be inhibited in this region; and in the tubificids where nearly all of the body is involved in the ascending gradient, head formation is impossible except at extreme anterior levels. In my opinion, however, the ascending gradient plays only a minor rôle in morphogenesis. I wish to point out that a similar series with regard to head formation can be found in the Turbellaria: *Planaria maculata* regenerates normal heads at all levels in reasonably short pieces; *P. dorocephala* in pieces of similar size forms normal heads at anterior levels only; *Dendrocoelum lacteum* will not produce heads at all behind the anterior third of the body; while in many polyclads head formation ceases posterior to the cephalic ganglia. In these forms, there is no posterior region of high rate to account for the facts, but the explanation lies in all probability in the character of the primary gradient, and in the rate at which new tissue grows out. The posterior rise in rate in oligochaetes is the expression of the increasing youth of cells in the posterior direction; it must be regarded as a secondary gradient superposed on the primary integrative gradient in the nervous system. Evidence for this point of view is found in the experiments on stimulation after section, where it was shown that these posterior regions of high rate are affected in the same way although not to the same extent as regions of low metabolic rate, and are, therefore, like the latter, subordinate parts dependent on correlation with more anterior regions. The fact, however, that they are stimulated to a less degree than the regions of low rate indicates that they possess a certain slight

amount of independence. One may say, then, that there is an antagonism between the primary and the secondary gradient, the one making for subordination of the posterior region, the other for independence. The primary gradient, however, always has the upper hand, for it is there from the very beginning, and each new segment which arises is forced, despite its high metabolic rate, to become part of the system of conduction, which has already been long established in the antero-posterior direction. The secondary gradient probably aids in the inhibition of head formation, but is unable otherwise to influence the morphogenetic effect of the primary gradient, unless the latter is eliminated by other factors. If this were not so, it would be impossible to understand why heads do not arise at the posterior end of long pieces of *Lumbriculus* and *Tubifex*. I might further point out that the zooids of the naids arise without regard to the secondary gradient, and often within it, and that the 'breaking' region of *Lumbriculus* is always within the secondary rise; both of which facts indicate that the secondary gradient offers very ineffective opposition to the primary gradient. For zooid formation is, as Child has shown ('11 a), a matter of physiological isolation from the primary gradient, usually as a result of decreased intensity of correlative stimulation, and would be inhibited by the presence of a gradient running in the other direction.

In how far do these conceptions, developed from the data on *Lumbriculus*, explain the facts of regeneration in the other forms? Why does *Dero* form normal heads at all levels? Experiments on the rate of metabolism of pieces of *Dero* at various times after section have shown that the rate is very high immediately after section, but begins to fall at once. Within three hours it has fallen below that of the corresponding part of the whole worm, in five hours it is still lower, and continues to fall until about twenty hours, after which a permanent and increasing rise in rate sets in. While I have not been able to devise any method for determining the time of head determination in *Dero*, yet it certainly is most improbable that it could occur during the very brief period of stimulation. One may therefore safely say that

the head of *Dero* is determined at a time when the rate of metabolism of the pieces is low, and that therefore normal heads are always produced.

Why does head formation cease at an extreme anterior level in the tubificids? To answer this question is one of the most difficult problems with which we have to deal in the field of morphogenesis. The dynamic factors which have thus far sufficed to explain the experimental results cannot be called upon here, for this result is independent of size of piece. Numerous attempts to alter the regenerative capacity of *Tubifex* and *Limnodrilus* have yielded negative results. The following suggestions are offered as to the cause of failure of head formation in these forms. In the first place, the new tissue grows out very slowly, and with a relatively low rate of metabolism. It therefore is very easily inhibited. Secondly, the secondary gradient runs far forward in these forms, and may serve as the inhibiting factor. The cells at the cut surface of a piece of *Tubifex* taken back of the fifteenth segment are in contact with a region of high rate, which is itself in contact with regions of higher rate, and so on. Unless, therefore, the cells at the surface grow out rapidly and with a very high rate, and this is contrary to fact, they could not dominate the regions behind. Heteromorphic tails would be expected under such conditions; they actually occur in the earthworms, but here the cells do not grow out fast enough, and they are inhibited before they have an opportunity to produce anything. It is obvious that this explanation can be tested experimentally, and I intend to continue my experiments with *Tubifex* in the hope of obtaining positive results. As to why the posterior end of such pieces does not give rise to heads has already been discussed; the secondary gradient cannot eliminate the primary gradient to that extent.

Regarding the formation of the posterior end, a word should be said. The cells at the posterior end grow out with a high rate of metabolism, but cannot become independent as long as the primary gradient persists. Owing to this gradient they are in contact with subordinate parts, and must develop in correlation with more anterior regions. If, however, the gradient is elimi-

nated, as can be done by taking very short pieces, or putting the pieces in depressing agents, then the cells at the posterior end can become independent, and develop a head. Biaxial heads result under these conditions. On the other hand, if the old piece has a high rate of metabolism, it may be able to dominate the new cells growing out at the cut surfaces to such an extent that neither one can produce a head but each develops a subordinate part, resulting in biaxial tails. In most organisms these occur in short pieces only, where the piece attains a high rate of metabolism through the stimulation from section. But in some oligochaetes, particularly the earthworm, biaxial tails are produced by long pieces. Dynamic factors alone cannot account for this result, but it is probably due to the presence of the secondary gradient.

The conception of the process of form regulation to which Child has come as the result of his extensive experiments with Coelenterates and flatworms can then be satisfactorily applied to the microdrilous oligochaetes. This conception may be briefly summarized as follows. The head can only arise if the cells which are to form it attain physiological isolation and independence from the rest of the piece. The head is a self-differentiating system; it does not develop in correlation with other parts but is the starting point for a new system of correlations, in other words, a new individual. In long pieces the head forms at the anterior end of the piece because the axial gradient determines that at this end alone can sufficient physiological isolation be attained. In short pieces, head formation depends on certain dynamic relations between the head-forming cells, and the old piece, and these dynamic relations are, in turn, dependent, although indirectly, on the axial gradient. All other parts are subordinate, and arise in correlation with the head, or with anterior regions, which themselves develop in correlation with the head. This statement is proven conclusively by the fact that no piece ever regenerates structures anterior to its level unless a head is formed first at its anterior end. The response of cells or groups of cells to isolation, physical or physiological, is the production of an apical region or head; this response is the

"fundamental reaction of the species," to use the phraseology of Child.

The apical region, or head region, or, in animals which develop a morphologically differentiated nervous system, the cephalic region of the nervous system which is the dominant part of the head is a closer approach than any other part of the organism to a morphological expression of this fundamental reaction system. The fundamental reaction system, dominance of the apical region and the axial gradient are all merely different aspects of the same general idea, viz., that the specific protoplasm of any organism consists fundamentally of a single physico-chemical reaction system. This system is the basis of inheritance and its dynamic capacities, the foundation of hereditary characters. The first step in organization and in embryonic development results from the establishment in one way or another, of some region or portion of this protoplasmic reaction system as a region of higher rate of dynamic activity. This region dominates development, becomes the apical or head region and determines the axial gradient or gradients which constitute the dynamic basis of polarity and of individuation. The organization and development of various parts of the organism rests upon a similar basis of fundamental reaction system and dominance and subordination of parts resulting from differences in rate of reaction (Child, '14 e).

V. SUMMARY

1. A gradient in rate of metabolism is demonstrated in the oligochaetes.

2. In the primary form of the gradient, the rate of metabolism is highest at the head and decreases along the antero-posterior axis. Among the oligochaetes this primary gradient is found only in *Aeolosoma*, and the zooids of the naids. The primary gradient is an integrative gradient.

3. In the other oligochaetes examined a posterior region of increased metabolic rate exists, and constitutes a secondary gradient superposed upon the primary gradient. The secondary gradient runs in the reversed direction from the primary; it results from the characteristic method of growth of annelids by continuous formation of new segments posteriorly, and is not integrative in character.

4. In *Dero limosa*, the secondary gradient involves the posterior third of the body; in *Lunbriculus inconstans*, it includes the posterior half of the body or more; and in the tubificids, it includes all of the body except the first five to fifteen segments.

5. In zooid formation, the gradient of the zooid gradually becomes independent of the gradient of the parent animal and is of the primary form. Owing to the processes of growth and dedifferentiation involved in zooid formation, the rate of metabolism of the fully developed zooid is higher than that of the parent; i.e., rejuvenescence results from asexual reproduction.

6. In oligochaetes a certain number of the most anterior segments are differentiated as a head.

7. In regeneration, the head and tail are replaced by outgrowth, the other parts by reorganization of the old tissue. No matter how many anterior segments are removed, only the typical number of head segments is, in general, replaced.

8. The head of oligochaetes will not regenerate a tail unless a certain number of trunk segments are included with it; nor will the end of the tail regenerate a head unless of a certain minimum size. Explanations of these facts are suggested.

9. In *Dero limosa*, any part of the body, whether long or short, regenerates a normal worm (with exceptions noted in 8).

10. In *Lumbriculus inconstans*, any part of the body, if of sufficient length, regenerates a normal worm. Short pieces show progressive inhibition of head formation along the axis; they give rise to anterior structures showing all gradations between a normal head and a normal tail. Normal posterior regeneration occurs at any level, and with any size of piece, but the number of segments regenerated decreases along the antero-posterior axis.

11. In *Tubifex*, head formation ceases at about the level of the fifteenth anterior segment, and, in *Limnodrilus*, at the level of the seventh segment, regardless of size of piece. Tail formation occurs at any level.

12. In *Lumbriculus inconstans*, it is determined, whether or not the head shall be normal within twenty to twenty-five hours after the pieces are cut.

13. The gradient of an axial series of pieces is not the same as that of a whole worm, because cutting stimulates. This stimulation is greater the shorter the piece and the lower its previous rate of metabolism. This stimulation is temporary, the time of

its duration varying with the different species, and is followed by a depression.

15. In *Lumbriculus inconstans*, short pieces from anterior regions are not much stimulated by section, and depression sets in within the time required for head determination. These pieces produce a high percentage of normal heads. Short posterior pieces are stimulated much more, and the stimulation lasts for a longer period of time, as long as the time required for head determination; these pieces produce a low percentage of normal heads, and a high percentage of inhibited structures.

16. The rate of metabolism of the piece during the time when the head is determined is, therefore, the important factor in anterior regeneration in short pieces. If the rate be high, then the region of new tissue which is to form the head is prevented from attaining the degree of independence and isolation necessary for normal head formation. Head formation will be inhibited in proportion to the metabolic rate of the old piece. On the other hand, if the metabolic rate of the old piece is low, then the new tissue suffers no inhibition and gives rise to a normal head. The dynamic relations set up between the old and the new tissue after cutting determine the character of the head, or whether a head shall form at all, or whether a tail shall form.

17. In long pieces, the dynamic factors are unimportant as the primary gradient determines that the cells at any anterior level are always more independent than those of a more posterior level. Therefore, normal heads are always formed on long pieces.

18. Experimental proof of the above conception is presented. If the rate of metabolism of the piece be depressed by means of cyanide, then the percentage of normal heads is increased; if the rate of the new tissue be decreased, then the percentage of normal heads decreases.

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